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MANUSCRIPT

Composite Biomarker Panel for Prediction of Severity and Diagnosis of Acute GVHD with T- Depleted Allogeneic Stem Cell Transplants- Single Centre Pilot Study

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Ethical approval: We have used the samples and clinical data on subjects recruited from the Kings Invasive Aspergillosis Anti-fungal study (REC no: 08/HA0808/154; R & D 08HA11; ClinicalTrials.gov No. NCT00816088).

Guarantor: RPV

Contributorship

The authors' contributions were as follows:

SSM: study design, collection of data, analyses of specimens and statistics including the panel, interpretation of data, drafting of the manuscript, critical review of the manuscript's content and approval of the final version submitted for publication;

VM: study design, interpretation of data, drafting of the manuscript, critical review of the manuscript's content and approval of the final version submitted for publication;

JC: study design, data collection, critical review of the manuscript's content and approval of the final version submitted for publication;

GFC, TD: Technical assistance on analyses of specimens, interpretation of data, critical review of the manuscript's content and approval of the final version submitted for publication;

AD: Statistic advice to develop biomarker panels, interpretation of data, critical review of the manuscript's content and approval of the final version submitted for publication;

VP, AP: study design, interpretation of data, critical review of the manuscript's content and approval of the final version submitted for publication;

MMC: study design, ethical approval for the previous anti-fungal study, collection of samples and data, interpretation of data, drafting of the manuscript, critical review of the manuscript's content and approval of the final version submitted for publication;

RS, RPV, TNB: study design, interpretation of data, drafting of the manuscript, critical review of the manuscript's content and approval of the final version submitted for publication

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Abstract

Aims: Acute Graft-versus-host disease (aGVHD) is a leading cause of morbidity and mortality following allogeneic haematopoietic stem cell transplantation (HSCT). The aim of this study was to evaluate the clinical utility of a composite biomarker panel to help identify individuals at risk of developing aGVHD, and to help predict and differentiate between severity of aGVHD following T-cell depleted allogeneic HSCT.

Methods: We retrospectively analyzed our cohort of biopsy confirmed aGVHD patients, who underwent T-cell deplete HSCT and matched them with negative controls without any evidence of aGVHD. Post-transplant serum samples on day 0,+7 and at onset of aGVHD were analyzed for Elafin, regenerating islet-derived 3-α (REG3α), soluble tumour necrosis factor receptor-1 (sTNFR1), soluble interleukin-2 receptor-α (sIL-2Rα) and hepatocyte growth factor (HGF). Biomarker data was combined as composite panels A-F (Table 2) using logistic regression analysis. Receiver Operating Characteristic (ROC) analysis was performed to study sensitivity and specificity of the composite panels.

Results: Our composite biomarker panels significantly differentiated between aGVHD and no GVHD patients at time of onset (Panel E*)* and reliably predicted severity of GVHD grades at Day 0 and 7 post-transplant (Panel B and D*)*. The area under the curve (AUC) for the composite panel at time of onset was 0.65 with specificity, sensitivity, positive and negative predictive values of 100%, 55.6%, 100% and 78.9%, respectively (*p=0.03*).

Conclusions: This pilot data supports the usefulness of these composite biomarker panels in the prediction of severity and diagnosis of acute GVHD in patients undergoing T-cell depleted reduced intensity allogeneic HSCT.

INTRODUCTION

Allogeneic haematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for a range of malignant and non-malignant haematological diseases. However, its use is limited by several complications including aberrant immune response by allo-reactive T-cells causing acute and chronic graft-versus-host disease (GVHD). The incidence of GVHD varies enormously from 10-80% depending on risk factors such as degree of human leukocyte antigen (HLA) disparity, graft source, conditioning regimen (standard myeloablative or reduced intensity; with or without T-cell depletion), CMV sero-status, recipient age, GVHD prophylaxis regimen, donor parity and sex mis-match[1–6].

The diagnosis of acute GVHD (aGVHD) remains mainly clinical, supplemented by biopsy where possible[7,8]. The skin is the most commonly involved organ and presentation can range from a limited maculopapular rash on the palms and soles to widespread skin involvement with muco-cutaneous ulceration and bullae formation. Similarly, other organs such as gastro-intestinal (GI) tract and liver can be involved and the symptoms range from mild to severe. Histology can be helpful but the findings are often non-specific[9].

Currently, there are no established biomarkers that can reliably diagnose, assess prognosis, or have any target organ-specificity of aGVHD[10,11]. However, important advances have been made in biomarker biology with potential clinical applications in aGVHD settings[11]. Hepatocyte growth factor (HGF) is a cytokine secreted by the mesenchymal cells as a physiological response to hepatic and intestinal damage and significantly higher concentrations were found in patients who developed severe aGVHD[12].

Soluble tumour necrosis factor alpha (TNFα) concentrations are higher in patients with aGVHD and positively correlated with its severity in some studies[13–16] whereas other studies did not support this relationship[5,13,17,18]. Soluble TNF receptor 1 (sTNFR1) is present in nanogram concentrations in a very stable state[13] and has been associated with severity of aGVHD in most studies[5,19,20]. Increased sTNFR1 at day 7 after HSCT was associated with the severity of GVHD and treatment related mortality[19,21].

Activated donor T cells express interleukin 2 receptor (IL-2R), which contains three subunits: α, β and γ, on their cell membrane[22,23]. Interleukin 2 (IL-2) binds to the β-subunit and is subsequently internalized and the α-subunit is shed from the cell surface and found in plasma as sIL-2Rα[23,24]. Increased sIL-2Rα concentrations were noted in aGVHD patients and closely correlated with GVHD severity[11,25,26].

Regenerating islet-derived 3-α (REG3α) is an antimicrobial protein secreted by Paneth cells[27] and a promising biomarker of lower GI aGVHD. Elafin is an elastase inhibitor overexpressed in inflamed epidermis[28,29] and is induced by inflammatory cytokines that mediate GVHD[20,30]. Increased elafin concentrations were noted at the onset of cutaneous aGVHD and closely correlated with aGVHD severity. It was also noted as a prognostic marker because of its association with non-relapse mortality (NRM) and overall survival (OS)[30].

These biomarkers have been studied in the context of T-replete allogeneic HSCT but data is lacking in the T-depleted setting. The aim of this study was to evaluate the clinical utility of composite biomarker panel consisting of HGF, Elafin, sIL-2Rα , sTNFR1, and REG3α in T-depleted allogeneic HSCT.

METHODS

Study population

This study is a retrospective analysis of a subset of allogeneic HSCT patients from the invasive aspergillosis study by Ceesay et al[31] (ClinicalTrials.gov No. NCT00816088; REC no: 08/HA0808/154) with full ethical approval. Patients were included if they had biopsy-confirmed diagnosis of aGVHD within 100 days of transplantation. These patients were then matched (age, sex, underlying haematological diagnosis, time since transplant, and conditioning regimen) with other allogeneic HSCT recipients who had no evidence of aGVHD. A total of 26 patients were included in the current study (12 confirmed aGVHD and 14 matched negative controls). Grading of aGVHD was according to Modified Seattle Glucksberg criteria[8].

Transplant conditioning protocols were either Alemtuzumab or Anti-thymocyte Globulin (ATG)-based (in vivo- T cell depletion) regimens. None of the patients had any evidence of infection at the time of sample collection.

Blood Samples and biomarker measurements

Serum samples at days 0 and 7 post-transplant and at the time of onset of aGVHD were evaluated for the biomarker panel (HGF, Elafin, sIL-2Rα , sTNFR1, and REG3α). HGF, sTNFR1 and sIL-2Rα were analysed using enzyme-linked immunosorbent assay (ELISA) methods from R&D Systems (Abingdon, Oxfordshire, UK). Elafin was measured using an ELISA method from Abcam (Cambridge, UK) and REG3α was measured using an ELISA method from Cloud-Clone (Yuhan, China). The assays for purpose of this study were carried out us-

ing ELISA plates for 5 biomarkers on three different days in duplicates with standard internal quality control (IQC) to limit inter-assay variation, corresponding to samples from day 0, day 7 post HSCT and at time of aGVHD onset in batches. The methods used for estimation of these biomarkers were internally validated before using them to measure clinical patients' samples. All patient samples were run in duplicate and coefficient of variance (CV) between duplicate samples was <10%. All method validation studies including precision, linearity, recovery, stability, carry over/under and lower limit of detection were duly carried out.

Each of these results performed in the study were reviewed by clinical biochemists following internal laboratory validation, median results were reviewed and correlated with clinical picture of the study patients following sample collection, although limited by retrospective nature of the study.

Statistical Analysis

Statistical analysis including ROC analysis was performed using *Analyse-It* version 2 (Leeds, UK). Data were tested for normality using the Shapiro-Wilk W test with a confidence interval of 95%. Patient characteristics were compared by chi-square test or Mann Whitney U test.

Logistic regression was performed using SigmaXL version 7 (Kitchener, Canada) to develop a composite panel of biomarkers (Panel A-F; Table 1). Binary logistic regression was used for a composite panel to discriminate between no GVHD and GVHD biopsy positive patients. Ordinal logistic regression was used to develop a composite panel for differentiating grading of GVHD. The best three markers with most significant p value were included to produce the best fit model.

Each equation of the composite panels differs based on different time-points of samples analysed, grade of aGVHD and dependent on statistically significant coefficient estimates of each panel. Numerical data were reported as median and inter-quartile range (IQR). A p value ≤ 0.05 was taken as statistically significant using logistical regression modelling.

To evaluate the reproducibility of the accuracy obtained in the composite panel and uncertainty around it, we conducted bootstrap (random subsampling from the same underlying population) cross-validation with 1000 replications to determine 95% confidence interval (CI) for the area under the curve (AUC).

Receiver Operating Characteristic curves (ROC) analysis was performed to evaluate the sensitivity and specificity of the composite panel at time of aGVHD onset. ROC curves at day 0 and 7 post-transplant could not be carried out because of the small sample size.

RESULTS

Baseline characteristics of aGVHD patients were similar to the negative controls (**Table 1**). All aGVHD cases had cutaneous involvement except one who had GI pathology (grade II) only**.** Six other patients had combined cutaneous and GI aGVHD (grade II-IV). The median time to aGVHD onset was 31 (range 12-77) days.

Table 1: Baseline characteristics of study population

Characteristic	aGVHD *N=12*	No GVHD N=14	*p* value
Sex, Male, n (%)	8 (66)	8 (57)	0.62
Age, median (IQR), years	50 (44-53)	53 (43-59)	0.41
Haematological diagnoses, n (%) Acute myeloid leukaemia Chronic myeloid leukaemia Aplastic anaemia Myeloproliferative neoplasm Myelodysplastic syndrome	 7 (58) 0 1 (8) 1 (8) 3 (25)	 8 (57) 1 (7) 2 (14) 0 3 (21)	0.68
Donor type, n (%) Related Unrelated	 3 (25) 9 (75)	 4 (29) 10 (71)	0.94
Donor HLA match, n (%) 10/10 9/10 8/10	 10 (83) 1 (8) 1 (8)	 12 (86) 1 (7) 1 (7)	0.98
Conditioning Intensity, n (%) Reduced intensity	12 (100)	14 (100)	0.92
T- cell depletion (in vivo), n (%) Alemtuzumab Anti-thymocyte Globulin (ATG)	 6 (50) 6 (50)	 8 (57) 6 (43)	0.72

Each of the composite biomarker panels were evaluated for their diagnostic utility of differentiating between no GVHD and biopsy positive aGVHD patients and correlate grading of disease severity on samples taken at day 0, 7 and time of onset of GVHD post-transplant.

Panel A (*Elafin* + *sIL-2R* + *sTNFR1*) and Panel C (*Elafin* + *HGF* + *REG3*) at day 0 and 7 post-transplant respectively could not differentiate between aGVHD and no GVHD cases. Composite panel E (*Elafin* + *sIL-2Rα* + *REG3α*) measured at on-set of aGVHD could differentiate between aGVHD and no GVHD patient groups (**Table 2**; **Panel A, C & E**).

Table 2: Comparison of composite biomarker panels (A-F) utility in diagnosis and predicting severity of aGVHD in T deplete HSCT patients. (Panels A-F; see supplementary for detail)

Panel ID	Patient cohort	Composite Biomarker Panel	Time of Sample	p value
Panel A	Non GVHD vs aGVHD	[1000x { 720.57 - (27.52 x Elafin) - (21.41 x sIL-2Rα) - (173.87x sTNFR1) }]	Day 0	0.54
Panel C	Non GVHD vs aGVHD	[1000x {471.75 + (48.60 x Elafin) - (20.21 x HGF) - (231.40x REG3α)}]	Day 7	0.85
Panel E	Non GVHD vs aGVHD	[1000x {(-39444) + (47.23 x Elafin) + (314.96 x sIL-2Rα) + (128.79x REG3α)}]	Onset of aGvHD	**0.02**
Panel B	Non GVHD vs Grade II	[1000x {constant - (86.55 x Elafin) + (1199 x sIL-2Rα) - (917.96x sTNFR1)}]	Day 0	0.20
	Non GvHD vs Grade III & IV			**0.01**
Panel D	Non-GVHD vs Grade II	[1000x {constant - (37.75 x Elafin) + (397.93 x HGF) - (29.67 x REG3α)}]	Day 7	0.14
	Non GvHD vs Grade III & IV			**0.02**
Panel F	Non GVHD vs Grade II	[1000x {constant - (33.63 x Elafin) - (247.78 x sIL-2Rα) - (212.29 x REG3α)}]	Onset of aGvHD	**<0.01**
	Non GvHD vs Grade III & IV			**<0.01**

The composite panel B and D differentiated between severity of aGVHD (Grade III-IV) and no GVHD patients at day 0 and 7 post-transplant respectively (p<0.01). Composite panel F also categorized the grading of aGVHD at time of onset; no aGVHD vs Grade I (p=0.02), no aGVHD vs Grade II (p<0.01), no aGVHD vs Grade III and IV aGVHD (p<0.01). (**Table 2**; **Panel B, D & F**).

ROC curve analysis was undertaken to evaluate specificity and sensitivity of this panel. The AUC for this panel at time of aGVHD onset was 0.73 (CI 50-70%, p=0.03) with specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) of 100%, 55.6%, 100% and 78.9% respectively, suggestive of a diagnostic utility for the panel (**Table 3 and Figure 1**).

Table 3: ROC curve analysis of biomarkers to diagnose GVHD in patients at time of onset of aGVHD symptoms

Bio-Markers	AUC	95% CI	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*p* value
Elafin	0.61	0.31-0.90	66.7	80.0	66.7	80.0	0.47
sIL-2Rα	0.57	0.30-0.84	55.6	80.0	62.5	75.0	0.62
REG3α	0.52	0.26-0.77	66.7	53.3	46.1	72.72	0.88
Composite panel E	0.73	0.48-0.99	55.6	100.0	100.0	78.94	**0.03**

AUC - area under curve; **CI** - confidence interval; **PPV**- positive predictive value; **NPV**- negative predictive value; **Composite panel E=** [1000x {(-39444) + (47.23 x Elafin) + (314.96 x sIL-2Rα) + (128.79x REG3α)}]

DISCUSSION

In our pilot study, we have demonstrated the usefulness of composite biomarker panels in the prediction of severity and diagnosis of aGVHD in patients undergoing T-cell depleted reduced intensity allogeneic HSCT. A number of small studies have investigated multiple proteins as individual potential biomarkers[20]; however none has been validated as a composite diagnostic panel or predictive laboratory test for aGVHD to date and there have been no validations of these biomarkers in the T-cell deplete HSCT. Chen *et al*[32] identified characteristics of candidate biomarkers that should allow (1) ease of testing, (2) a widely available technique with good reproducibility, (3) relatively low cost, (4) adequate sensitivity with high specificity, (5) predictive value, (6) correlation with severity and (7) correlation with treatment response.

There are only few published papers focusing on a composite biomarker panel for aGVHD and most were studied in non-T deplete allograft patients. Paczesny *et al* (2009)[20] developed a four biomarker panel using HGF, sIL-2Rα, IL8 and sTNFR1 with potential diagnostic utility in patients at onset of aGVHD and provide prognostic information independent of aGVHD severity. August *et al* (2011)[33] reported that a panel of three biomarkers including sIL-2 Rα, sTNFR1 and soluble CD8 is the best screening test with an AUC of 0.77 at Day 15 post-transplant. Levine *et al* (2015)[34] developed the Ann Arbor scoring system based on a composite panel using ST2 (suppression of tumorigenicity-2), sTNFR1 and REG3α with potential to predict the development of gastrointestinal aGVHD. Simultaneous use of several biomarkers may increase specificity and hence diagnostic and/or predictive values for aGVHD. Combination of tissue-specific and

systemic biomarkers is likely to be more informative than single biomarkers for aGVHD diagnosis.

The utility of composite panel in HSCT patients is further evident by ROC curve analysis of composite biomarkers in Panel E with specificity of 100% and sensitivity of 55.6%, at time of aGVHD onset. Depending on specific clinical situation, the sensitivity and specificity of the panel can be adjusted using ROC curve analysis and reference ranges for composite biomarkers could be derived[33]. For example, the sensitivity of the panel improves to 66.7% with specificity of 86.7% providing PPV of 75.0% and NPV of 81.2% for diagnosis (**Figure 1**). Thus, composite biomarkers in Panel E, measured at time of onset of aGVHD, significantly differentiated between no GVHD and biopsy positive aGVHD group (all grades) in contrast to Panels A and C measured at day 0 and 7 post HSCT. This highlights its potential use as an alternative diagnostic tool for aGVHD with an added convenience of non-invasive sampling to patients.

Promisingly, composite biomarker Panel B and Panel D analyzed at day 0 and 7 post-transplant in our pilot study were also able to predict severity of Grades III-IV aGVHD before onset. These panels could potentially serve as an important laboratory tool for pre-emptive modification of immunosuppressive therapy at an earlier stage and reduce associated morbidity with severe aGVHD. Our combined biomarker Panel F was also able to accurately predict between all grades of aGVHD at time of onset, which could be useful for identifying potential low risk patients (mild-moderate aGVHD) and predict prognosis from aGVHD related morbidity.

Very limited evidence exists for evaluation of similar biomarkers in patients with 'biopsy proven' aGVHD and previous studies predominantly included patients who received non-manipulated or T-replete allogeneic HSCT. T cell depletion with drugs like alemtuzumab results in subdued immune responses to inflammation with increase in homeostatic regulatory T cells (Treg) and decrease in pro-inflammatory cytokines[35]. Any biomarker assay based on pro-inflammatory proteins in this setting may not correlate well with diagnosis of aGVHD in theory, thus forming our basis of null hypothesis for this study. This is the first reported study within T-depleted allogeneic HSCT settings and despite the potential anti-inflammatory effects of the conditioning regimen, we could reliably report potential clinical utility of a composite biomarker panel in diagnosis of aGVHD in these patients, who otherwise required biopsy for diagnosis.

From an economical perspective, Elafin and REG3α costs €21 per test and sTNFR1, HGF, sIL-2Rα analysis cost €14 per test in our centre. Therefore, a composite panel using three biomarkers will cost around €55 which is considerably cheaper and cost-effective than combined costs of diagnostic tissue biopsy (operator time and skills, tissue processing & reporting); while removing, the risks associated with invasive procedures in this immunocompromised patient population. Currently these assays are available as research only tests in our centre, but performed by state registered biomedical scientists working in NHS laboratories, however not accredited or part of any EQA exercise or sample exchange program. The use of these assays, once validated in larger cohort of clinical samples in a prospective study, can be implemented by most accredited laboratories familiar with automated ELISA methods run by state registered appropriately trained staff.

The assay is limited by its relatively labour intensive technique and need for minimum number of samples, which can potentially dictate frequency of samples run in clinical laboratory practice. This can be reduced by full automation and performed in larger regional diagnostic reference centers for cost effective operation.

Our study is however limited by the small number of patients. Due to the retrospective nature of this study, we could not study composite biomarker trends to assess any correlation with severity of disease, subsequent response to therapy and its impact on survival. This would be an interesting research question in a larger prospective study and could help answer its usefulness in serial measurements of biomarkers to help guide withdrawal of immunosuppression.

Conclusion

The use of composite panels is more useful than individual markers. This is again demonstrated with panels of composite biomarker proposed in this pilot study, providing an improvement in the sensitivity and specificity of diagnosis of aGVHD as well as predicting disease severity in T deplete HSCT. Larger studies are still required to validate their findings and assess its potential impact on non-relapse mortality (NRM) and overall survival (OS) with early aGVHD diagnosis.

Take Home Messages (Key Points):

1. Acute Graft versus host disease (aGVHD) is an unpredictable and potentially debilitating complication of allogeneic stem cell transplants (HSCT). No validated diagnostic blood test for aGVHD currently exists, although multiple blood proteins have been described as potential biomarkers of aGVHD, mainly in HSCT treated with T-cell replete conditioning regimens.
2. Composite serum biomarker panels developed using logistic regression modelling of HGF, Elafin, sIL-2Rα , sTNFR1, and REG3α, in this retrospective pilot study of T-cell depleted HSCTs reported for the first time, successfully predicted severe aGVHD at early time points of Day 0 and Day 7 post-transplant and diagnosed onset of acute GVHD with a high positive and negative predictive value.
3. The use of composite panels is more useful than individual markers. Once validated in larger prospective studies, these composite biomarker panels can be potentially used as an alternative diagnostic tool for aGVHD, with an added convenience of cost effective non-invasive sampling, and an important laboratory tool for pre-emptive modification of immunosuppressive therapy at an earlier stage and reduce associated morbidity with severe aGVHD.

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Legends:

Figure 1: ROC curve analysis of composite biomarker Panel E:

The area under curve (AUC) result for the composite panel at time of onset of aGVHD is 73%, with specificity of 100% and sensitivity of 55.6% (CI 50-70%, p=0.03). The sensitivity of the panel improves to 66.7% with specificity of 86.7% with positive predictive value (PPV) of 75.0% and negative predictive value (NPV) of 81.2% for diagnosis.

Abbreviations:

aGVHD - acute Graft vs Host disease; ROC- Receiver operating characteristics

NPV - Negative predictive value; PPV- Positive predictive value

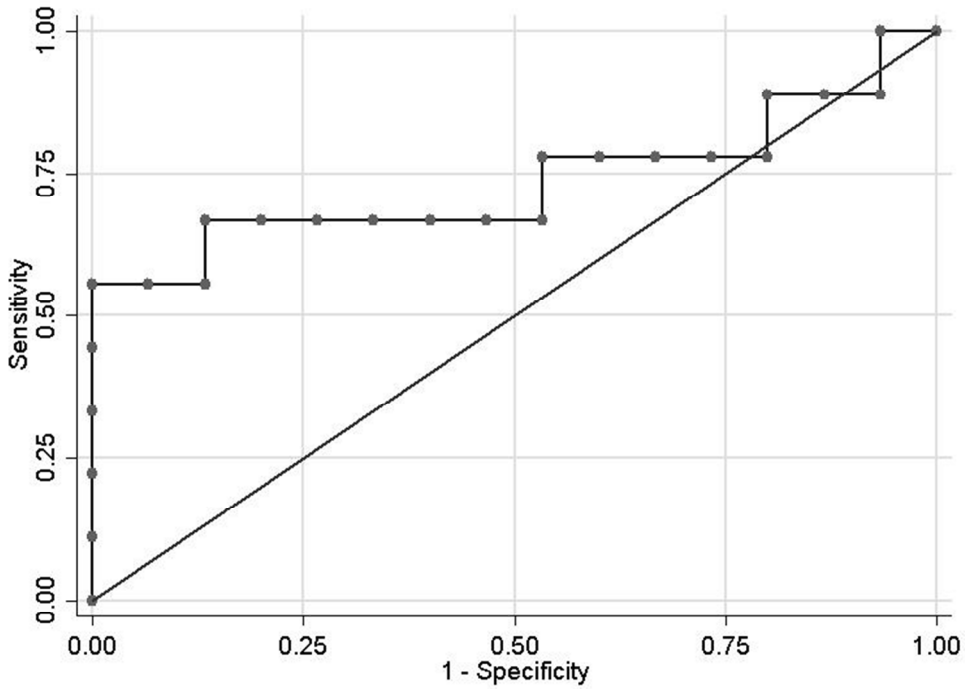

Figure 1: ROC curve analysis of composite biomarker Panel E
Figure 1
65x47mm (300 x 300 DPI)

SUPPLEMENTARY DATA

Table A: Composite Panel A between no GVHD and aGVHD biopsy positive at day 0 post-transplant

Biomarkers	Coefficient Estimate x 10^{-3}	Standard error x 10^{-3}	Z value	*P* value
Composite panel A	720.57	1173	0.61	0.54
Elafin	-27.52	41.88	-0.66	0.51
sIL-2Rα*	-21.41	284.86	-0.08	0.94
sTNFR1**	-173.87	405.56	-0.43	0.67

Composite panel A = [1000x { 720.57 - (27.52 x Elafin) - (21.41 x sIL-2Rα) - (173.87x sTNFR1) }]

*sIL-2Rα: Soluble Interleukin-2 Receptor-α

**sTNFR1: Soluble Tumour Necrosis Factor Receptor 1

Table B: Composite Panel B between groups by GVHD grading in aGVHD biopsy positive group at Day 0 post-transplant

Biomarkers	Coefficient Estimate x 10^{-3}	Standard error x 10^{-3}	Z value	*P* value
No GVHD Vs Grade I	413.02 (constant 1)	937.61	0.44	0.65
No GVHD Vs Grade II	1257 (constant 2)	982.19	1.28	0.20
No GvHD Vs Grade III and IV	2665 (constant 3)	1098.78	2.42	**0.01**
Individual biomarkers using ordinal logistic regression as best fit model				
Elafin	-86.55	60.22	-1.43	0.15
sIL-2Rα*	1199	668.80	1.79	0.07
sTNFR1**	-917.96	711.32	-1.29	0.19

Composite panel B= [1000x {constant - (86.55 x Elafin) + (1199 x sIL-2Rα) - (917.96x sTNFR1)}]

*sIL-2Rα: Soluble Interleukin-2 Receptor-α

**sTNFR1: Soluble Tumour Necrosis Factor Receptor 1

Table C: Composite Panel C between non-GVHD and aGVHD biopsy positive at Day 7 post-transplant

Biomarkers	Coefficient Estimate x 10^{-3}	Standard error x 10^{-3}	Z value	*P* Value
Composite panel C	471.75	2593	0.18	0.85
Elafin	48.60	33.41	1.45	0.14
HGF*	-20.21	561.88	-0.03	0.97
REG3α**	-231.40	278.48	-0.83	0.40

Composite panel C = [1000x {471.75 + (48.60 x Elafin) - (20.21 x HGF) - (231.40x REG3α)}]

*HGF: Hepatocyte Growth Factor

**REG3α: Regenerating islet-derived 3-α

Table D : Composite Panel D between groups by grading in aGVHD biopsy positive group at Day 7 post-transplant

Biomarkers	Coefficient Estimate x 10^{-3}	Standard error x 10^{-3}	Z value	*P* Value
No GVHD Vs Grade 1	778.98 (constant 1)	1071	0.79	0.46
No GVHD Vs Grade 2	1611 (constant 2)	1112	1.45	0.14
No GvHD Vs Grade 3 and 4	2691 (constant 3)	1236	2.13	**0.02**
Individual biomarkers using ordinal logistic regression as best fit model				
Elafin	-37.75	21.76	-1.73	0.08
HGF*	397.93	556.02	0.71	0.47
REG3α**	-29.67	58.96	-1.01	0.31

Composite panel D = [1000x {constant - (37.75 x Elafin) + (397.93 x HGF) - (29.67 x REG3α)}]

*HGF: Hepatocyte Growth Factor.

**REG3α: Regenerating islet-derived 3-α

Table E : Composite Panel E between non-GVHD and aGVHD biopsy positive at time of onset of aGVHD

Biomarkers	Coefficient Estimate x 10^{-3}	Standard error x 10^{-3}	*Z* value	*P* Value
Composite panel E	-39444	1698	-2.32	**0.02**
Elafin	47.23	28.44	1.66	0.09
sIL-2Rα*	314.96	232.33	1.35	0.17
REG3α**	128.79	99.62	1.29	0.19

Composite panel E = [1000x {(-39444) + (47.23 x Elafin) + (314.96 x sIL-2Rα) + (128.79x REG3α)}]

*sIL-2Rα: Soluble Interleukin-2 Receptor-α

**REG3α: Regenerating islet-derived 3-α

Table F : Composite Panel F between groups by grading in aGVHD biopsy positive group at time of onset of aGVHD

Biomarkers	Coefficient Estimate x 10^{-3}	Standard error x 10^{-3}	Z value	*P* Value
Non- GVHD Vs Grade 1	3797 (constant 1)	1699	2.23	**0.02**
Non-GVHD Vs Grade 2	4904 (constant 2)	1852	2.64	**<0.01**
Non- GVHD Vs Grade 3 and 4	6167 (constant 3)	2048	3.01	**<0.01**
Individual biomarkers using ordinal logistic regression as best fit model				
Elafin	-33.63	18.09	-1.85	0.06
sIL-2Rα*	-247.78	209.24	-1.18	0.23
REG3α**	-212.29	155.14	-1.36	0.17

Composite panel F= [1000x {constant - (33.63 x Elafin) - (247.78 x sIL-2Rα) - (212.29 x REG3α)}]

*sIL-2Rα: Soluble Interleukin-2 Receptor-α

**REG3α: Regenerating islet-derived 3-α

Grading Criteria of Acute Graft-versus-Host Disease

(Modified Glucksberg criteria)

Stage	Skin	Liver	GI Tract
I	Maculopapular rash <25% of body surface area (BSA)	Bilirubin 35-50µm/l	<1000ml diarrhoea/day; Nausea/Vomiting; anorexia
II	Maculopapular rash 25-50% of BSA	Bilirubin 51-100µm/l	1000mL-1500mL diarrhoea/day
III	Maculopapular rash >50% BSA or generalized erythroderma	Bilirubin 101-250µm/l	>1500mL diarrhoea/day
IV	Generalized erythroderma with bullous formation and desquamation	Bilirubin >250µm/l	Severe abdominal pain with or without ileus

Overall acute GVHD Grade

Grade	Skin Stage	Liver Stage	Gut Stage
I	1-2	0	0
II	1-3	1	1
III	2-3	2-3	2-4
IV	4	4	